pH-Sensitive Hydrogel Based On A Novel Photocross-Linkable Copolymer

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A pH sensitive hydrogel has been prepared by a UV irradiation technique. Starting polymer was the PHM (poly hydroxyethylaspartamide methacrylated) obtained from polyaspartamide (PHEA) partially derivatized with methacrylic anhydride (MA). This new copolymer has been further derivatized with succinic anhydride (SA) to obtain PHM-SA that has been cross-linked by UV irradiation to form a pH sensitive hydrogel. The network, recovered after washing as a powder, has been been characterized by FT-IR spectrophotometry and particle size distribution analysis. Moreover, to have information about water affinity of the prepared sample, swelling measurements have been carried out in aqueous media mimicking biological fluids. The possibility to employ the prepared hydrogel as a pH-sensitive drug delivery system (DDS) has been investigated. In particular, ibuprofen ((*S*)(+)4-isobutyl-α-methylphenyl-acetic acid), chosen as a model drug, has been entrapped into the PHM-SA hydrogel, and in vitro release studies have showed that its release rate depends on different swelling of the network as a function of the environmental pH.

Introduction

In recent years, special attention has been paid to hydrogels for biomedical and pharmaceutical applications. In fact, hydrogels seem to be a promising class of modern biomaterials for the development of devices usable in therapy and diagnosis. The biocompatibility of these materials makes them very attractive as biomaterials and drug carriers. Infact, their application includes drug delivery systems, sensors, cell-incorporating networks, and hybrid artificial organs.[1−4]

Hydrogels can be divided into two categories: (1) conventional hydrogels, which are usually uncharged and exhibit no significant changes in swelling with change of the external environment, and (2) stimulus-responsive hydrogels that exhibit large volume changes in response to small changes in pH, temperature, electric field, and light.[5] The stimulus-sensitive behavior of these hydrogels can be used for biosensors and environment-responsive drug release.[4,6] The pH-sensitive behavior of hydrogels is due to the presence in the polymeric chains of ionizable groups which are charged or not depending on the surrounding condition. As a consequence, changes in the hydrogen concentration in the surrounding fluid can bring about an abrupt change in the swelling. These pH-responsive hydrogels can be divided into anionic and cationic catagories, according to the typology of ionizable groups. Anionic hydrogels are based on polymers containing carboxyl or sulfonic groups which ionize above their pK_a value. These hydrogels swell at high pH due to the electrostatic repulsion between the anionic groups formed along the chains.[7,8] Cationic hydrogels, on the other hand, contain amine groups that become charged at low pH.[9,10] Thus, their swelling behavior is opposite of that showed by anionic hydrogels: at a high pH value, the pendant amine groups are relatively hydrophobic and a low swelling occurs; when pH decreases below the pK_a of the ionizable group, the resulting formation of positive charges along the polymeric backbone causes an increase in the swelling.

There are a number of different chemical and physical methods used for the formation of permanent hydrogels;[11] however, the synthesis by radiation seems to be the most useful method for medical purposes. In particular, UV-induced cross-linking of hydrophilic polymers is a practical method for producing well-defined networks, and it provides significant advantages over conventional chemical cross-linking, such as the easiness (the synthesis is often carried out in a single step and without the presence of initiators), safety, and low cost.[12] However, despite these advantages, there are few reports on the preparation of hydrogels via photocross-linking of water-soluble polymers.[13−17]

In this paper, we describe the preparation and characterization of a pH-sensitive network based on a new ionizable photosensitive copolymer indicated as PHM-SA. PHM-SA copolymer was obtained by partial modification of PHM with succinic anhydride (SA), where PHM is a derivative of α,β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide (PHEA) with methacrylic anhydride (MA).

PHM-SA is a water-soluble photosensitive copolymer, carrying methacrylate groups in the side chain and carboxyl groups which allow it to confer a pH-sensitive behavior to the obtained hydrogels.

Due to the known reactivity of methacrylate groups toward radical reactions, an aqueous solution of PHM-SA has been irradiated at 313 nm for 2 h thus obtaining a cross-linked

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material that has been recovered in a microparticulate shape. The obtained microparticles are able to swell in aqueous media, and their physicochemical characterization is here reported.

In addition, the potential use of the PHM-SA hydrogel as a novel drug delivery system has been investigated by using ibuprofen as a model drug. Ibuprofen is a nonsteroidal antiinflammatory drug (NSAID) that reduces the production of hormones responsible for inflammation and pain in the body. It is used to reduce the temperature, pain, inflammation, and stiffness caused by many conditions, such as osteoarthritis, rheumatoid arthritis, primary dysmenorrhea, and other conditions. Hydrogels designed to release ibuprofen in a prolonged and/or controlled way provide potentially many advantages, such as an optimization of pharmacokinetics and a consequent reduction in the side effects, such as gastric irritation and ulcer. For these reasons, ibuprofen has been entrapped into the PHM-SA hydrogel, and the drug release rate has been evaluated by performing in vitro studies in simulated gastric and intestinal fluids.

Materials and Methods

Chemicals. All reagents were of analytical grade, unless otherwise stated. D,L-aspartic acid, ethanolamine, *N,N*-dimethylformamide (DMF), anhydrous *N,N*-dimethylacetamide (DMA), methacrylic anhydride (MA), succinic anhydride (SA), and triethylamine (TEA) were from Fluka (Italy). Ibuprofen ((*S*)(+)4-isobutyl-α-methylphenyl-acetic acid, 99%) and D_2O (isotopic purity 99.9%) were purchased from Aldrich Chemical Co. (Italy). Diethyl ether, acetone, tetrahydrofuran, acetonitrile, acetic acid, and 2-propanol were purchased from Merck (Germany).

α,β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide (PHEA) was prepared by reaction of a polysuccinimide (PSI), obtained by thermal polycondensation of D,L-aspartic acid, with ethanolamine in DMF solution, purified, and characterized according to a procedure reported elsewhere.[18] The batch of PHEA used in the present study has a weight-average molecular weight of 40 kDa (M_w/M_n 1.74), determined by size exclusion chromatography (SEC) analysis.

Apparatus. Molecular weights of PHEA, PHM, and PHM-SA were determined by a SEC system equipped with a pump system, two Phenogel columns from Phenomenex (5μm particle size, 10^3 Å and 10^4 Å of pores size), and a 410 differential refractometer (DRI) as concentration detector, all from Waters (Mildford, MA). The molecular weights were determined by using PEO/PEG as standards (range 4000−318000 Da), DMF + 0.01M LiBr as mobile phase; 50 °C and a low of 0.8 mL/min.

^{1}H NMR (D_2O) and ^{13}C NMR (DMSO-d_6) spectra were obtained with a Bruker AC-250 instrument.

FT-IR spectra were recorded as pellets in KBr in the range 4000−400 cm^{-1} using a Perkin-Elmer 1720 Fourier Transform Spectrophotometer with a resolution of 1 cm^{-1}; each spectrum was recorded after 100 scans.

UV irradiation was performed by using a Rayonet reactor equipped with a Rayonet Carousel motor assembly and 16 mercury lamps of 8 W at medium pressure with an emission at 313 nm.

Centrifugations were performed with an International Equipment Company Centra MP4R equipped with an 854 rotor and temperature control.

Particle size distribution was studied by using an image processing and analysis system Leica Quantimet Q 500 equipped with a Leica Wild 3 D stereomicroscope. This image processor calculates the particle area and converts it to equivalent circle diameter.

Gas chromatography was performed by using a Star C × 3400 Varian chromatograph.

X-ray diffraction analysis was performed by using a diffractometer Philips PW 1729 X-ray generator diffractometer. The experimental parameters were set as follows: Cu Kα radiation, tube setting 40 KV, 20 mA; angular speed 2° (2 θ/min); range recorded 10−60° (2 θ/min); time costant 1 s, chart speed 2 cm/min.

Release studies were performed in a Benchtop 80 °C Incubator Orbital Shaker model 420.

High-pressure liquid chromatography (HPLC) analyses were carried out by using an Agilent 1100 Liquid Chromatograph equipped with a Rheodyne 7125 injector (fitted with a 20 μL loop) and an Agilent 1100 HPLC detector on line with a computerized workstation. Column: reversed-phase C_{18} (μ Bondapak; 10 μm of 250 × 4.6 mm internal diameter, obtained from Waters). Mobile phase: CH_3CN/CH_3COOH (5 g/L), 50:50, flow 1 mL/min, λ 264 nm.

PHM Synthesis. Derivatization of PHEA with methacrylic anhydride to obtain PHM copolymer was carried out in the organic phase (anhydrous DMA), by using TEA as catalyst, according to the following procedure: 2 g of PHEA were dissolved in 40 mL of anhydrous *N,N*-dimethylacetamide (DMA), and then a suitable amount of triethylamine (TEA) and methacrylic anhydride (MA) was added, according to $X = 0.5$ and $Y = 0.5$, being X = moles of methacrylic anhydride/moles of PHEA repeating unit and Y = moles of TEA/moles of methacrylic anhydride.

The reaction was kept at 40 °C under continuous stirring for 48 h. After this time, the reaction mixture was precipitated in 400 mL of 2-propanol and centrifugated for 10 min, at 11 800 rpm and 4 °C. The product was recovered, washed several times with 2-propanol (4 × 40 mL) and acetone (4 × 40 mL), and then dried under vacuum.

PHM copolymer thus obtained was dissolved in 200 mL of double-distilled water and subjected to extensive dialysis by using Visking Dialysis Tubing (18/32 in.) with a molecular weight cutoff of 12 000−14 000. After dialysis, the solution was dried by freeze-drying. PHM was obtained with a yield of 97−99% (w/w), based on the starting PHEA.

Characterization of PHM Copolymer. FT-IR spectrum (KBr) showed a broad band centered at 3300 cm^{-1} (ν_{as} OH + ν_{as} NH + ν_{as} NH_2); bands at 1717 (ν_{as} COO); 1656 (amide I); 1543 (amide II), 1300 (scissoring −C=C−), 1170 (ν_s COO) and 950 (wagging −C=C−) cm^{-1}.

^{1}H NMR spectrum (D_2O) showed: δ 1.9 (s, 3H, −CO−C(**CH_3**)=CH_2), 2.81 (m, 2H, −CH−**CH_2**−CO−NH), 3.39 (t, 2H, −NH−**CH_2**−CH_2−O−), 3.57 (m, 2H, −O−**CH_2**−CH(OH) −CH_2-O-), 3.68 (t, 2H, −NH−CH_2−**CH_2**−O−), 4.28 (m, 1H, −O−CH_2−**CH**(OH)−CH_2−), 4.55-4.81 (m,

3H, $-CH(OH)-\mathbf{CH_2}-O-CO-$, $-NH-\mathbf{CH}(CO)-CH_2-$), 5.72 and 6.12 (2s, 2H, $-CO-C(CH_3)=\mathbf{CH_2}$).

The degree of derivatization (DD) was determined by ^{1}H NMR and calculated by the following ratio:

$$DD = (\text{methacrylic groups/polymer repeating unit}) \times 100$$

In particular, DD was calculated by comparing the integral of the peaks related to protons at 1.9 δ as well as to protons between 5.72 and 6.12 δ respectively assigned to $-CO-C(\mathbf{CH_3})=CH_2$ and $-CO-C(CH_3)=\mathbf{CH_2}$ (belonging to linked MA) with the integral related to protons at 2.81 δ awardable to $-CH-\mathbf{CH_2}-CO-NH$ (belonging to PHEA). The degree of derivatization was expressed as mean value and resulted to be 30 ± 1 mol %. The weight-average molecular weight of PHM copolymer determined by SEC measurements was 46.9 kDa (M_w/M_n 1.78).

^{13}C NMR spectrum (DMSO-d_6) showed: δ 171.4 and 169.6 (C=O amidic), 166.6 (C=O ester), 135.7 ($-CH_2=$), 126.1 ($-C=CH_2$), 62.5 ($-CH_2-OH$), 59.6 (—CH—), 49.9 (CH_2-NH) and 18.0 ($CH_3-C=CH_2$).

Derivatization of PHM with Succinic Anhydride. Derivatization of PHM with succinic anhydride was carried out in the organic phase (anhydrous DMA), by using TEA as catalyst, according to the following procedure. 500 mg of PHM were dissolved in 8 mL of anhydrous *N*,*N*-dimethylacetamide (DMA), and then suitable amounts of triethylamine (TEA) and a solution of succinic anhydride (SA) in DMA were added, according to $X^1 = 0.5$ and $Y^1 = 0.2$, with X^1 = moles of succinic anhydride/moles of PHEA repeating unit and Y^1 = moles of TEA/moles of succinic anhydride.

The reaction was kept at 25 °C under continuous stirring for 24 h. After this time, the reaction mixture was precipitated in 100 mL of THF/Et_2O 1:1 and centrifugated for 15 min, at 11 800 rpm and 4 °C. The product was recovered, washed several times with ethanol/acetone 2:1 (6 × 40 mL) and acetone (1 × 40 mL), and then dried under vacuum.

The PHM-SA copolymer thus obtained was dissolved in 100 mL of double-distilled water and subjected to extensive dialysis by using Visking Dialysis Tubing (18/32 in.) with a molecular weight cutoff of 12 000−14 000. After dialysis, the solution was dried by freeze-drying. PHM-SA was obtained with a yield of 97−98% (w/w), based on the starting PHM.

Characterization of the PHM-SA Copolymer. The FT-IR spectrum (KBr) showed a broad band centered at 3300 cm^{-1} (ν_{as} OH + ν_{as} NH + ν_{as} NH_2), a weak band at 2541 (ν_{as} OH), and bands at 1725 (ν_{as} COO); 1654 (amide I), 1541 (amide II), 1300 (scissoring —C=C—), 1170 (ν_s COO), and 950 (wagging —C=C—) cm^{-1}.

^{1}H NMR spectrum (D_2O) showed: δ 1.9 (s, 3H, $-CO-C(\mathbf{CH_3})=CH_2$), 2.47 (d, 4H, $-CO-\mathbf{CH_2}-\mathbf{CH_2}-COOH$) 2.81 (m, 2H, $-CH-\mathbf{CH_2}-CO-NH$), 3.39 (t, 2H, $-NH-\mathbf{CH_2}-CH_2-O-$), 3.57 (m, 2H, $-O-\mathbf{CH_2}-CH(OH)-CH_2-O-$), 3.68 (t, 2H, $-NH-CH_2-\mathbf{CH_2}-O-$), 4.28 (m, 1H, $-O-CH_2-\mathbf{CH}(OH)-CH_2-$), 4.55-4.81 (m, 3H, $-CH(OH)-\mathbf{CH_2}-O-CO-$, $-NH-\mathbf{CH}(CO)-CH_2-$), 5.72 and 6.12 (2s, 2H, $-CO-C(CH_3)=\mathbf{CH_2}$).

The degree of derivatization (DD) was determined by ^{1}HNMR and calculated by the following ratio:

$$DD = (\text{succinic groups/polymer repeating unit}) \times 100$$

In particular, DD was calculated by comparing the integral of the peaks related to protons at 2.47 δ assigned to $-CO-\mathbf{CH_2}-\mathbf{CH_2}-COOH$ (belonging to linked SA) with the integral related to protons at 2.81 δ awardable to $-CH-\mathbf{CH_2}-CO-NH$ (belonging to PHEA). The degree of derivatization was expressed as mean value and resulted to be 35 ± 1 mol %. The weight-average molecular weight of the PHM-SA copolymer determined by SEC measurements was 44.6 kDa (M_w/M_n 1.81).

^{13}C NMR spectrum (DMSO-d_6) showed: δ 172.5 (—COOH), 171.4 and 169.6 (C=O amidic), 166.6 (C=O ester), 135.7 ($-CH_2=$), 126.1 ($-C=CH_2$), 62.5 ($-CH_2-OH$), 59.6 (—CH—), 49.9 (CH_2-NH), 29.1 ($-CO-CH_2-CH_2-CO-$) and 18.0 ($CH_3-C=CH_2$).

Preparation of Polymeric Networks of PHM-SA. A solution of PHM-SA (60 mg/mL) in double-distilled water was placed in Pyrex tubes each equipped with an internal Pyrex piston in order to have a sample of about 2 mm in thickness, and then they were irradiated for 2 h under argon at 313 nm.

After 2 h of irradiation, a wall-to-wall product was obtained that was purified by several washes with double-distilled water and centrifuging, from time to time, at 12 000 rpm, and 4 °C for 20 min. After further washing with acetone, a powder was obtained that was dried at 10^{-1} mmHg in the presence of P_2O_5 until its weight remained constant. In this way, a microparticulate sample has been obtained.

Swelling Studies. The swelling ability of PHM-SA microparticles was determined at 37 °C in double-distilled water, HCl 0.1N (pH 1.0, simulated gastric fluid), and phosphate buffer (KH_2PO_4, Na_2HPO_4) at pH 6.8 (simulated intestinal fluid).

In particular, aliquots of the dried sample were exactly weighed and brought into contact with the penetrant medium until the equilibrium swelling was reached, and then each swollen sample was filtered, plugged with blotting paper, and weighed. The weight swelling ratio (q) was calculated as follows:

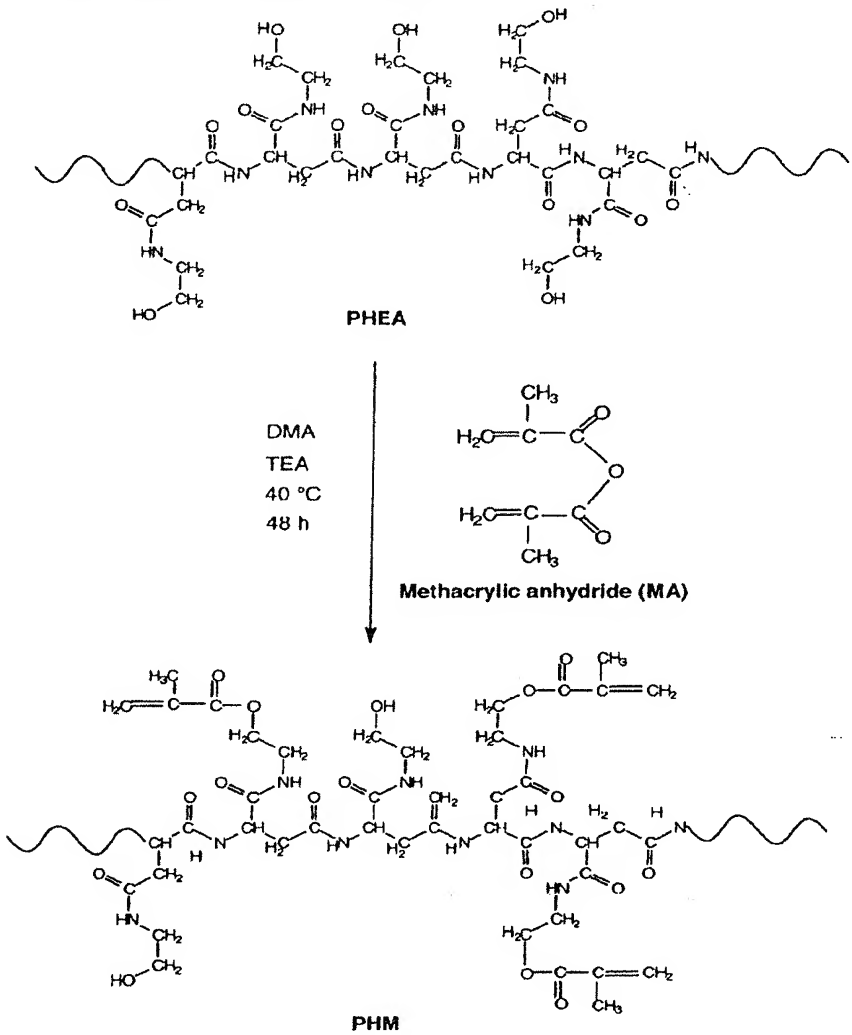
$$q = W_s/W_d$$

where W_s and W_d are the weights of swollen and dry sample, respectively.

Each experiment was performed in triplicate and the results were in agreement within ±2% error.

Chemical Hydrolysis. Chemical hydrolysis of PHM-SA hydrogel was investigated in pH 1.0 HCl and pH 6.8 phosphate buffer solutions. Each sample (30 mg) was dispersed and maintained under continuous stirring (100 rpm) in (i) 10 mL of pH 1.0 HCl at 37.0 ± 0.1 °C for 2 h or (iii) 10 mL of pH 6.8 phosphate buffer at 37.0 ± 0.1 °C for 24 h. After the hydrolysis time, the samples were neutralized (when pH 1.0 solution has been employed) and centrifuged at 12 000 rpm at 4 °C for 15 min, and the supernatant was separated. For each sample, the remaining hydrogel was washed 5 times with distilled water under continuous stirring

Scheme 1. Reaction of PHEA with Methacrylic Anhydride to Give PHM



for 1 h to extract soluble polymer degradation products and electrolytes entrapped in the network. Finally, the hydrogel was washed with acetone and centrifuged at 12 000 rpm at 4 °C for 15 min. The recovered solid residue was dried to a constant weight and characterized by swelling studies in double-distilled water. Each experiment was performed in triplicate and the results were in agreement within ±2% error.

Drug Lading by Soaking Procedure. A concentrated solution of ibuprofen in methanol was added to PHM-SA microparticles. The mixture was maintained at room temperature with stirring for 3 days. After this time, the solvent was removed by filtration, and the sample was rapidly washed with methanol in order to remove exteriorized ibuprofen. Drug loaded microparticles thus prepared were dried at 10^{-1} mmHg in the presence of P_2O_5 until constant weight. The complete removal of methanol has been confirmed by gas chromatography.

Determination of Drug Amount Entrapped in PHM-SA Microparticles. 50 mg of drug loaded PHM-SA microparticles were extensively extracted at room temperature with 60 mL of methanol. The liquids of extraction were collected and evaporated under vacuum at 40 °C. The obtained residue, dissolved in methanol, was assayed by HPLC for the quantitative determination of ibuprofen. The amount of drug entrapped in PHM-SA microparticles was found to be 20% w/w.

Drug Release at pH 1.0 and 6.8 from PHM-SA Microparticles. Aliquots (10 mg) of the drug loaded PHM-SA microparticles were dispersed in flasks containing HCl 0.1 N (pH 1.0, simulated gastric fluid) and maintained at 37 °C ± 0.1 °C in an incubator for 2 h (100 rpm). Since the drug release was not complete after 2 h of incubation at pH 1.0, a solution of 0.2 M tribasic sodium phosphate/NaOH 1N was added to raise the pH to 6.8 (simulated intestinal fluid), according to the method reported in USP XXII (drug-release test, method A for enteric-coated particles). Then the experiment was continued until 24 h. Sink conditions were maintained throughout the experiment. Then, at suitable time intervals, samples were filtered and analyzed by HPLC. Each

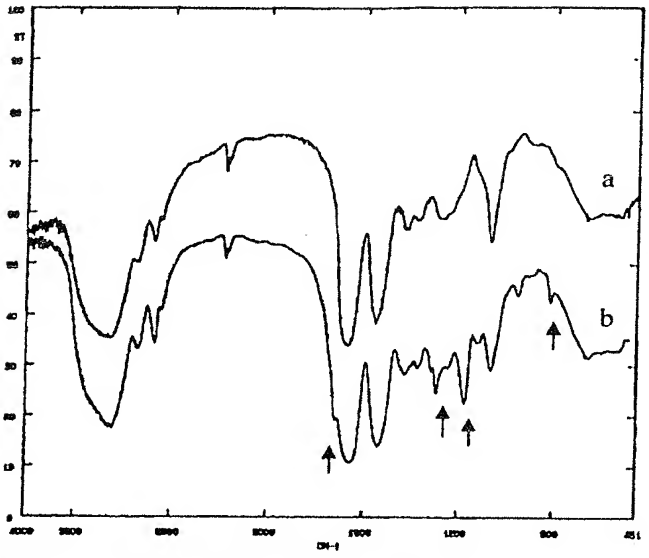


Figure 1. FT-IR spectra of (a) PHEA and (b) PHM.

experiment was carried out in triplicate, and the results were in agreement within ±5% standard error.

Results and Discussion

Considering that pH responsive systems are very attractive as biomaterials and drug carriers, the aim of this study has been the synthesis of a novel ionizable hydrogel able to show a different swelling behavior as a function of the environmental pH.

We have employed, as the starting material, a new copolymer PHM, obtained by partial derivatization of α,β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide (PHEA) with methacrylic anhydride (MA). The derivatization of PHEA with MA has been performed in the organic phase (anhydrous DMA), for 48 h at 40 °C, by using TEA as catalyst. The reaction yield is almost quantitative. The reaction of PHEA with MA is shown in Scheme 1. This reaction is a useful method to introduce, in a simple manner, pendent double bonds and ester groups in side chains of starting PHEA. The presence of double bonds provides reactivity toward radical reaction activated by UV rays, whereas ester groups confer a potential biodegradability to hydrogels based on this copolymer.

FT-IR analysis of PHM, compared to the PHEA one, showed new bands due to the introduction of MA residues. As reported in Figure 1 (spectrum b), the most important signals, assigned to MA residues, are (i) a band at 1717 cm^{-1} due to COO asymmetric stretching of ester group, (ii) two bands of the vinilydene CH deformation at 1300 and 950 cm^{-1} due to scissoring and wagging moves respectively, and (iii) a band at 1170 cm^{-1} due to COO symmetric stretching of ester group.

^1H NMR and ^{13}C NMR spectra of the PHM copolymer (Figure 2) have confirmed the presence of methacrylate groups in the copolymer. The degree of derivatization of PHM, calculated as reported in the Experimental Section, resulted to be 30 ± 1 mol %.

In a successive step, PHM has been partially functionalized with succinic anhydride (SA) to obtain the new ionizable copolymer PHM-SA. The derivatization of the PHM copolymer with SA has been performed in the organic phase (anhydrous DMA), for 24 h at 25 °C, by using TEA as catalyst. The reaction yield was almost quantitative. This reaction allowed us to introduce carboxyl pendent groups in the polymeric backbone which confer to the new copolymer the ability to ionize or not as a function of the external pH. The reaction of PHM with SA is shown in Scheme 2.

FT-IR analysis of PHM-SA, compared to the PHM one, showed the presence of a band at 1725 cm^{-1} due to the introduction of carboxyl groups of SA as well as to the presence of ester bonds of linked MA and SA. Obviously, the FT-IR spectra shows also the bands at 1300 (scissoring —C=C—) and 950 cm^{-1} (wagging —C=C-) due to methacrylate residues.

^1H NMR and ^{13}C NMR spectra of PHM-SA copolymer (Figure 3a,b) have confirmed the presence of the succinic groups in the copolymer. The degree of derivatization of

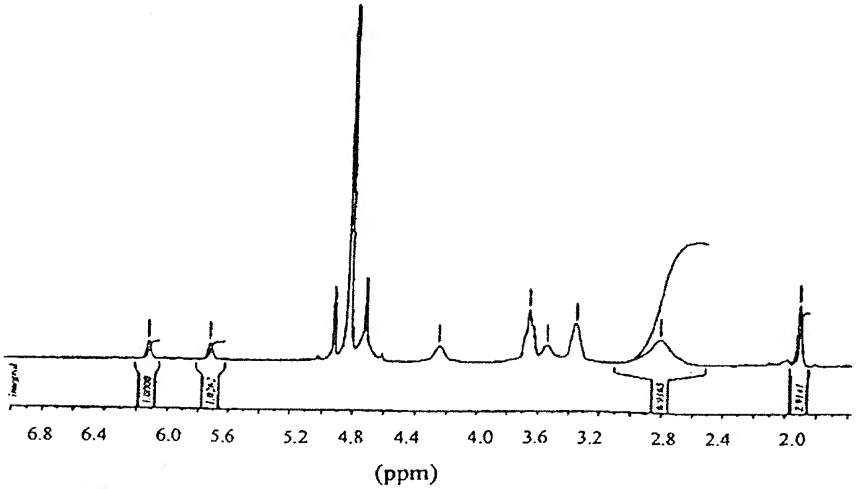
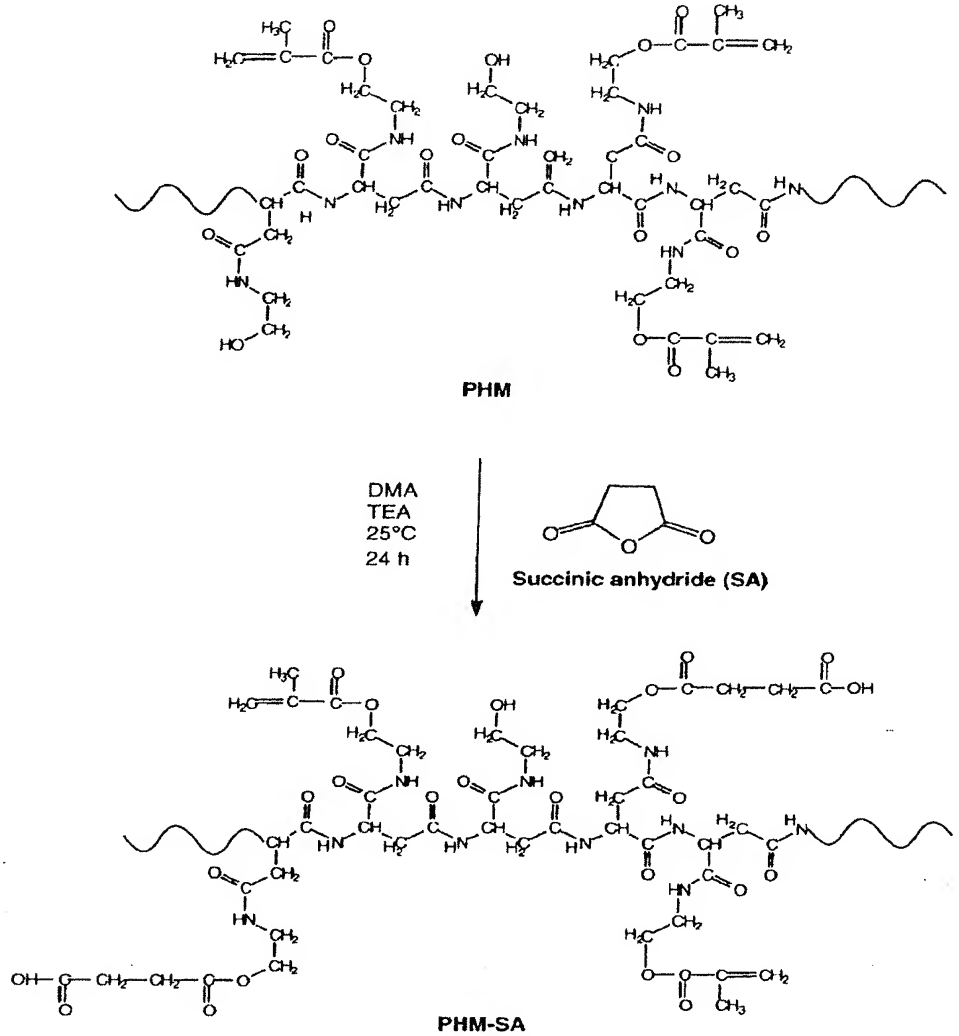


Figure 2. ^1H NMR spectrum of PHM in D_2O.

Scheme 2. Reaction of PHM with Succinic Anhydride to Give PHM-SA



PHM-SA, calculated as reported in the Experimental Section, was found to be 35 ± 1 mol %.

To prepare a hydrogel with a potential pH sensitive behavior, an aqueous solution of PHM-SA has been exposed at 313 nm for 2 h under argon, without the presence of chemical initiators. In fact, in PHM-SA, there are methacrylate residues (chromophore groups) very reactive toward UV rays, and then, after UV irradiation, the formation of a biradical (through a $\pi \rightarrow \pi^*$ transition) in the vinyl group could give rise to a radical cross-linking in accordance with radical polymerization of vinyl molecules.[19] Due to the presence in PHM-SA chains of several chromophore groups in the excited state, it is possible to obtain a cross-linked structure without the use of photoinitiators. On the other hand, it is well-known that photoinitiators are reactive molecules (such as benzophenone, acetophenone, and 2,2-dimethoxy-2-phenylacetophenone) whose traces can cause toxic effects on humans; therefore, various authors have prepared hydrogels via rapid photopolymerization in the absence of photoinitiators.[12,13,20] As a consequence, the possibility to obtain hydrogels in the absence of chemical initiators gives the opportunity to confer biocompatibility to the obtained systems.

Scheme 3 reports a schematic representation of the polymeric network obtained when PHM-SA is irradiated with UV rays.

The sample, obtained as a wall-to-wall network has been purified by several washes with double-distilled water. After a further washing with acetone, a powder has been obtained and dried as described in the experimental part. Microparticles so obtained have been characterized as far as their physicochemical properties.

They were insoluble in water and in common organic solvents, such as dichloromethane, acetone, ethanol, dimethyl sulfoxide, dimethylacetamide, and dimethylformamide.

FT-IR analysis has confirmed that the cross-linking reaction happened. In fact, the FT-IR spectrum of the PHM-SA network shows different peaks in comparison with starting PHM-SA before irradiation (Figure 4). In particular, the more interesting feature is the complete disappearance

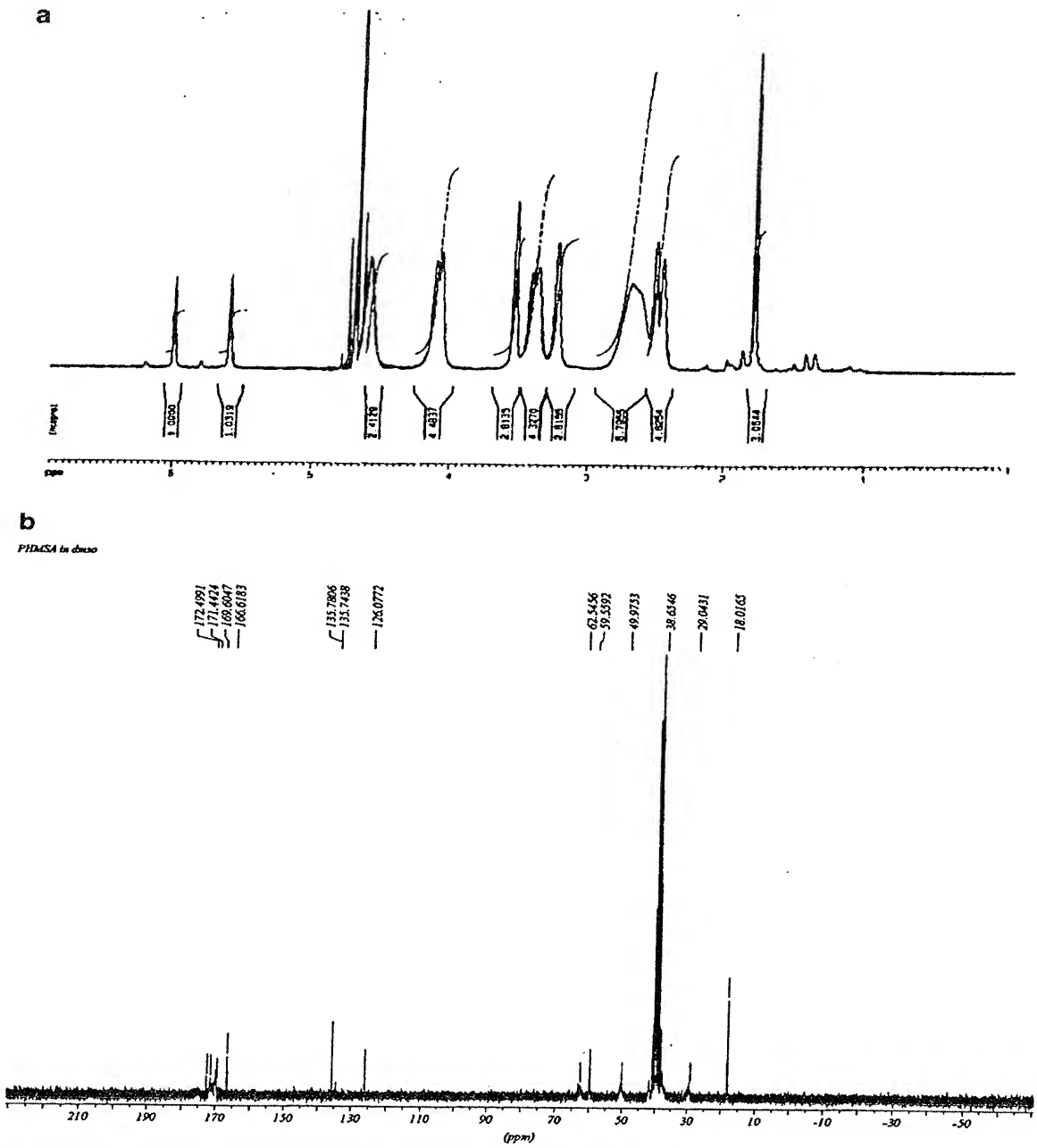


Figure 3. (a) ^{1}H NMR spectrum of PHM-SA in D_2O. (b) ^{13}C NMR spectrum of PHM-SA in DMSO-d_6.

of peaks related to double bonds, i.e., 1300 (scissoring —C=C—) and 950 cm^{-1} (wagging —C=C—), assigned to the vinyl group of the methacrylate residues in PHM-SA. This suggests that the cross-linking reaction induced by UV rays involves the opening of double bonds, probably through the formation of free radicals which give rise to inter- and intrapolymeric chain cross-linked bonds. In addition, after irradiation, the shift of the asymmetric stretching of the ester group from 1725 to about 1730 cm^{-1} confirms the lack of conjugation with the double bonds of methacrylate residues in PHM-SA.

Microparticles have been also characterized as far as dimensional analysis is concerned. A typical particle size distribution of PHM-SA microparticles is shown in Figure 5.

It can be observed that there is an asymmetric particle distribution with a maximum value of equivalent diameter in the range 5−10 μm. This particle size distribution appears suitable for a potential control of drug release rate as reported elsewhere for similar microparticles.[21]

In addition, to evaluate the swelling ability of PHM-SA microparticles, these were characterized by swelling studies in various aqueous media, mimicking some physiological fluids such as HCl 0.1 N solution (pH 1.0, simulated gastric juice) and phosphate buffer solution pH 6.8 (simulated

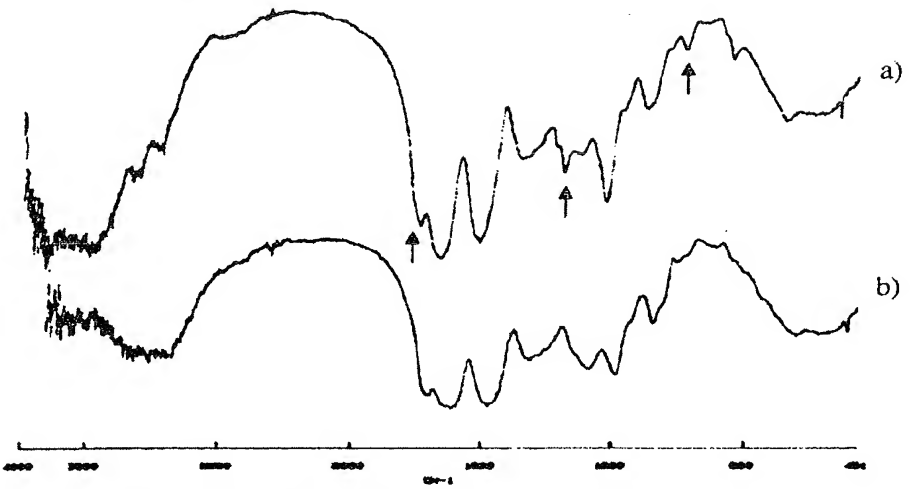
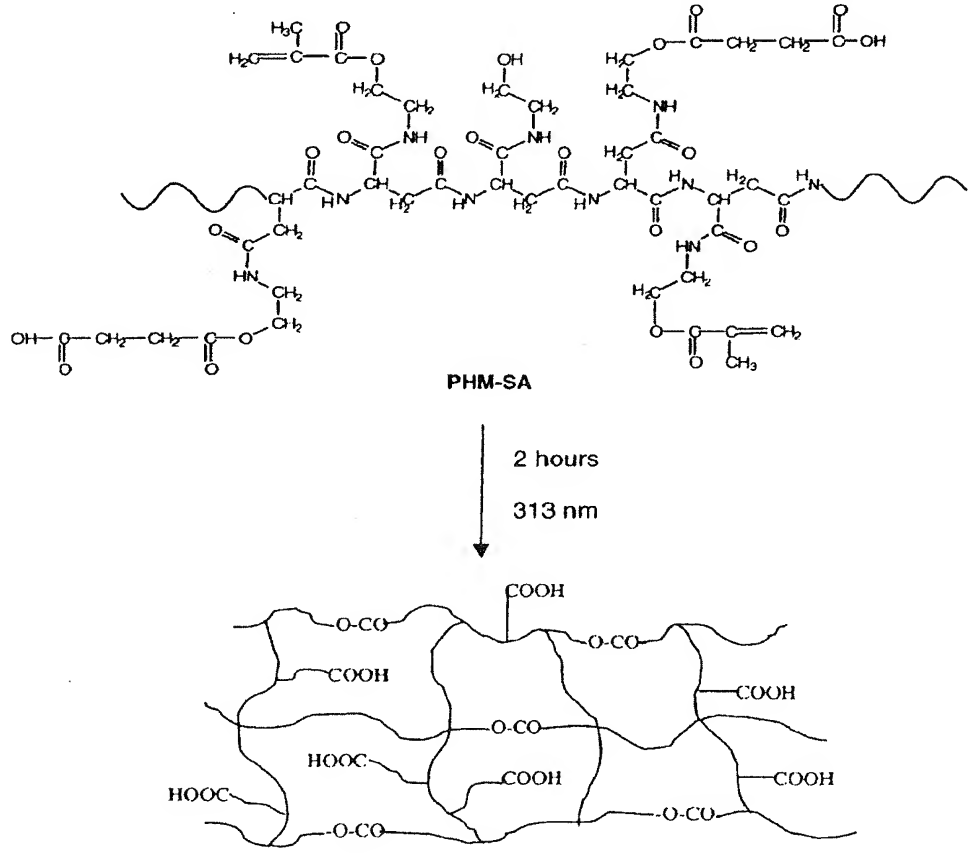


Figure 4. FT-IR spectra of (a) starting un-cross-linked PHM-SA and (b) cross-linked PHM-SA.

Scheme 3. Schematic Representation of the Polymeric Network Based on PHM-SA Obtained by UV-Irradiation



intestinal fluid) besides double-distilled water. Swelling data in terms of q are reported in Table 1.

All of the results evidenced a remarkable affinity of the PHM-SA microparticles toward the aqueous media depending on the pH of the medium.

It is evident, as expected, that at pH 1.0 there is a considerable lowering of the q value due to the presence of pendant acidic groups, undissociated at this pH value. When the pH is 6.8, the water regain is greater, and then q increases, as a consequence of electrostatic repulsions between polymeric chains due to the increase of dissociated groups at this pH value. However, the value of q at pH 6.8 is lower than that found in double-distilled water due to the ionic strength and the osmotic pressure of the first medium.

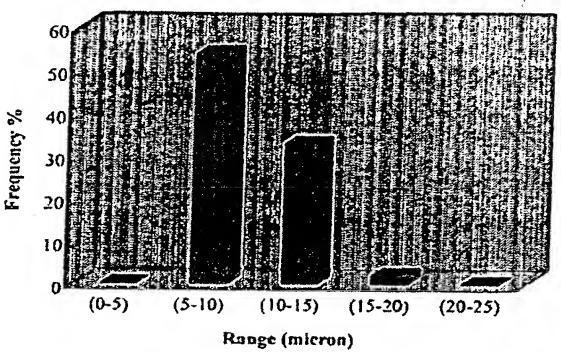


Figure 5. Size distribution profile of microparticles based on cross-linked PHM-SA.

Table 1. Swelling Degree of PHM-SA Microparticles, Expressed as *q* Value at pH 1.0, Phosphate Buffer Solution pH 6.8, and Double-Distilled Water

	q = Ws/Wd		
sample	pH 1.0	pH 6.8	double-distilled water
PHM-SA microparticles	4.5 ± 0.1	14.0 ± 0.2	33.5 ± 0.4

Table 2. Yield (%) and Swelling Degree (Expressed as *q* Value) in Double-Distilled Water after Chemical Hydrolysis of PHM-SA Hydrogel at pH 1.0 for 2 h and pH 6.8 for 24 h at 37.0 ± 0.1 °C

treatment	yield (%) after hydrolysis	*q* value in double-distilled water after hydrolysis
sample incubated at pH 1.0 for 2 hours	97.5 ± 2.0	35.1 ± 0.6
sample incubated at pH 6.8 for 24 hours	98.2 ± 1.7	34.6 ± 0.5

Taking into account the presence of ester and peptide groups in the chemical structure of thye PHM-SA hydrogel, both potentially degradable, chemical hydrolysis studies have been performed under simulated gastrointestinal conditions. In particular, the sample was treated at 37.0 ± 0.1 °C at pH 1.0 for 2 h (to simulate gastric fluid) and at pH 6.8 for 24 h (to simulate intestinal fluid). After this time, the percent residual weight was determined and the recovered sample has been characterized by swelling studies in double-distilled water. These results are reported in Table 2.

The high yield of the sample recovered after this treatment as well as the slight increase of swelling suggest that the PHM-SA hydrogel undergos a negligible degradation under the investigated conditions.

The remarkable affinity toward the aqueous media and the pH-dependent swelling allow us to suppose a potential ability of the prepared hydrogel to entrap drug molecules and to release them in a physiological medium owing to pH variation.

To confirm this supposition, PHM-SA microparticles have been loaded with ibuprofen, chosen as a model drug. The drug loading has been carried out by soaking the microparticles in a concentrated drug solution, by using methanol as solvent. Since PHM-SA microparticles swell also in methanol, this allows the drug molecules to diffuse into swollen

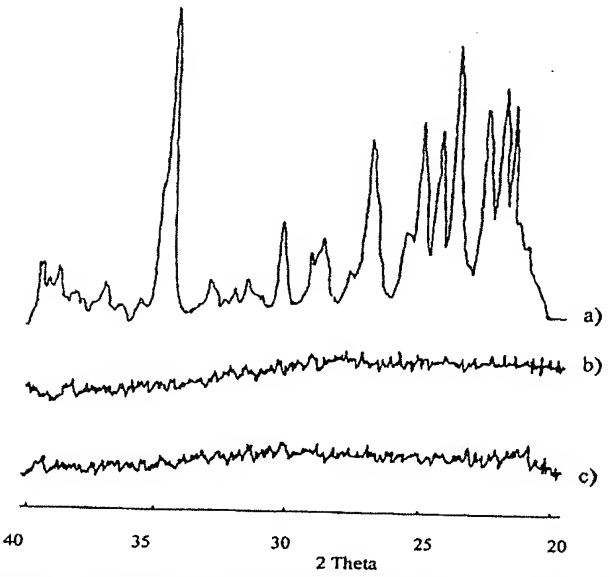


Figure 6. X-ray diffraction patterns of ibuprofen (a), unloaded PHM-SA microparticles (b), and drug loaded PHM-SA microparticles (c).

polymeric network thus obtaining an effective drug entrapment (drug loading 20% w/w).

The determination of the drug dispersion state in the PHM-SA sample was performed by X-ray analysis. Figure 6 reports the X-ray diffraction patterns of pure ibuprofen and unloaded and ibuprofen loaded PHM-SA microparticles. It is evident that pure ibuprofen is in the crystalline state, whereas when it is entrapped in PHM-SA microparticles, it is in an amorphous state. Drug unloaded PHM-SA microparticles result also in an amorphous structure. The obtained data demonstrate that, during the cross-linking reaction, no crystalline region was formed and that the drug is molecularly entrapped inside the network, i.e., in a physical state readily available to the dissolution process in a release medium.

The possibility to employ the prepared sample as a pH-sensitive DDS, has been investigated by in vitro release studies under experimental conditions which simulate gastrointestinal fluids. The experiments have been carried out at 37 °C at pH 1.0 (simulated gastric fluid) and pH 6.8 (simulated intestinal fluid) by using the pH change method (see the Experimental Section).

Figure 7 depicts drug release, expressed as the percent of drug (related to the entrapped total dose) delivered as a function of time. The same figure reports also the dissolution profile of free drug (corresponding to the amount loaded in the hydrogel) in gastrointestinal simulated fluids (pH 1.0/6.8).

It is possible to observe that at pH 1.0, after 2 h, 83% of free drug is dissolved and when the pH jumps to 6.8 the complete dissolution is quickly reached.

A different profile has been found when ibuprofen is released from the PHM-SA hydrogel. In fact, it is possible to observe a remarkable variation in the amount of drug released when the pH changes from 1.0 to 6.8. In particular, the release of ibuprofen is quite low at pH 1 (only 7% of ibuprofen is released after 2 h at this pH value), whereas it increases quickly when the pH jumps to 6.8. Then, a bimodal

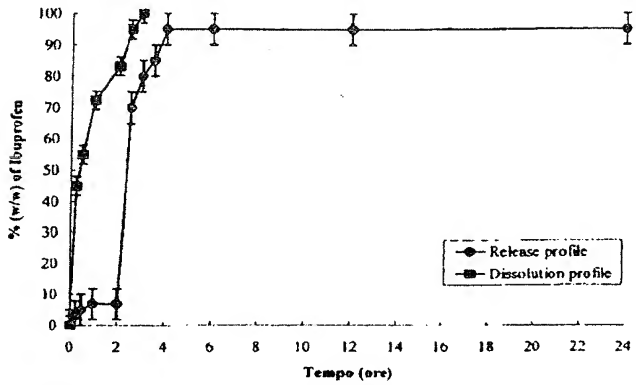


Figure 7. Ibuprofen dissolution and release from PHM-SA microparticles at pH 1.0 from 0 to 2 h and pH 6.8 from 2 to 24 h by pH change method at 37 °C.

release pattern occurs for ibuprofen with a slow process at a low pH followed by a quick one in the simulated intestinal juice.These data, in comparison with those obtained for the dissolution of free ibuprofen, confirm that the drug release is strongly affected by matrix, because of its pH sensitive properties, i.e., the difference in rate of release between pH 1.0 and 6.8 can be attributed to a change in swelling of the hydrogel. On the other hand, the minimal chemical degradation of the network which occurs under simulated gastrointestinal conditions has a negligible effect on drug release.

Therefore, the obtained results show that the prepared sample acts evidently as a pH-sensitive hydrogel, since it shows a swelling behavior that is influenced by the pH value of the penetrating media. According to the release data, PHM-SA microparticles seem to be particularly suitable for the release of nonsteroidal antiinflammatory agents in the intestinal tract, thus ensuring a reduction of topical damage to the gastric mucosa. In addition, the small size of PHM-SA microparticles containing ibuprofen could allow the distribution in a wide area of the gastrointestinal tract, thus avoiding located concentrations and improving oral tolerability.

Conclusions

A novel copolymer at protein-like structure containing double bonds and acid residues has been synthesized and characterized. This copolymer, indicated as PHM-SA, gives rise to a cross-linked structure owing to UV irradiation. The obtained transparent hydrogel has been purified, dried, and shaped as microparticles. PHM-SA microparticles show a narrow size distribution profile and a great affinity toward aqueous media, simulating physiological fluids. In particular, PHM-SA microparticles due to their chemical structure show a swelling dependent on the environmental pH, being lower in simulated gastric juice and higher in simulated intestinal fluid. The possibility to employ these microparticles as pH sensitive drug delivery systems has been evaluated by loading the sample with ibuprofen, chosen as a model drug. Experimental release data suggest that PHM-SA microparticles could be ideal candidates for the release in the intestinal tract of nonsteroidal antiinflammatory drugs thus reducing ulcerogenic effects on the gastric mucosa.

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References and Notes

(1) Brinkman, E.; Van der Does, L.; Banties, A. *Biomaterials* **1991**, *12*, 63.
(2) Gombotz, W. R.; Hoffman, A. S. In *Hydrogels in Medicine and Pharmacy, Vol. I. Fundamentals*; Peppas, N. A., Ed.; CRC Press: Boca Raton, FL, 1987; pp 95−126.
(3) Ulbrich, K.; Subr, S.; Seymour, L. W.; Duncan, R. *J. Controlled Release* **1993**, *24*, 181.
(4) Bell, C. L.; Peppas, N. A. *Biomaterials* **1996**, *17*, 1203.
(5) Rosiak, J. M.; Yoshii, F. *Nucl. Instrum. Methods Phys. Res. B*, **1999**, *151*, 56.
(6) Vaidya, R.; Wilkins, E. *Med. Eng. Phys.* **1994**, *16*, 416.
(7) Khare, A. R.; Peppas, N. A. *Biomaterials* **1995**, *16*, 559.
(8) Soppimath, K. S.; Kulkami, A. R.; Aminabhavi, T. M. *J. Controlled Release* **2001**, *75*, 331.
(9) Siegel, R. A.; Firestone, B. A.; Johannes, I.; Cornejo, *J. Polym. Prepr.* **1993**, *34* (1), 231.
(10) Hariharan, D.; Peppas, N. A. *Polymer* **1996**, *37*, 149.
(11) Peppas, N. A.; Mikos, A. G. In *Hydrogels in Medicine and Pharmacy, vol I. Fundamentals*; Peppas, N. A., Ed.; CRC Press: Boca Raton, FL, 1987; pp 1−25.
(12) Nakayama, Y.; Matsuda, T. *J. Polym. Sci. Part A Polym. Chem.* **1992**, *30*, 2451.
(13) Andreopoulos, F. M.; Deible, C. R.; Stauffer, M. T.; Weber, S. G.; Wagner, W. R.; Beckman, E. J.; Russell, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 6235.
(14) Vyavahare, N.; Kohn, J. *J. Polym. Sci. Part A Polym. Chem.* **1994**, *32*, 1271.
(15) Doytcheva, M.; Dotcheva, D.; Stamenova, R.; Orahovats, A.; Tsvetanov, C.; Leder, J. *J. Appl. Polym. Sci.* **1997**, *64*, 2299.
(16) Muhlebach, A.; Muller, B.; Pharisa, C.; Hofmann, M.; Seiferling, B.; Guerry, D. *J. Polym. Sci. Part A Polym. Chem.* **1997**, *35*, 3603.
(17) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581
(18) Giammona, G.; Carlisi, B.; Palazzo, S. *J. Polym. Sci. Polym. Chem.* **1987**, *25*, 2813.
(19) Barltrop, J. A.; Coyle, J. D. *Excited States in Organic Chemistry*; Wiley: London, 1975.
(20) Nakayama, Y.; Matsuda, T. *J. Polym. Sci. Part A Polym. Chem.* **1993**, *31*, 977.
(21) Cavallaro, G.; Giammona G.; Iemma, F.; Mazzalupo, R.; Trombino, S.; Picci, N.; Pitarresi, G. *Colloid Polym. Sci.* **2001**, *279*, 688.

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